SCREENING OF PHYTOCHEMICALS OF LOCALLY AVAILABLE SELECTED WEEDS FOR THE BIOCONTROL OF FUNGAL DISEASES OF LEAVES OF *Luffa acutangula* AND *Luffa cylindrica*

A

REVISED SYNOPSIS

Submitted for Registration

In The

Maharshi Dayanand Saraswati University,

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To work as a candidate for Ph.D Degree

(Faculty of Science)

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1. Name (In English) : Deepika Chittoriya

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2. Title of the Research Work

(In English) : Screening of phytochemicals of locally available selected weeds for the biocontrol of fungal diseases of leaf of *Luffa acutangula* and *Luffa cylindrica*.

(In Hindi) :

3. Location

a. Institution/Department : Where the work is to be Done **Department of Botany, Government College of Ajmer, Rajasthan**

b. Geographical area of Investigation, : Ajmer (Rajasthan)

Contents

1. **Introduction**
2. **Importance of proposed investigation**
3. **Objectives**
4. **Reviews (work already done on the subject of proposed research study)**
5. **Research gaps identified in the proposed field of investigation**
6. **Major Hypothesis**
7. **Research Methodology**
8. **List of References cited**

Introduction

Vegetables are one of the most natural foods that contain different vitamins, minerals and thousands of other plant chemicals known to provide health benefits. Vegetables have generous amounts of vitamins. Vitamins regulate metabolism and also used to convert the fats and carbohydrates into energy. Green vegetables include varying amounts of photochemical such as lutein and indoles, and have potential antioxidant and health promoting benefits.

Cucurbitaceae is the giant family of plants possessing medicinal properties. It is the gourd family of flowering plants, belonging to the order Cucurbitales and containing 118 genera and 845 species of food and ornamental plants. The most important plants of this family, cultivated in temperate areas are *Luffa acutangula* and *Luffa cylindrica*.

In Rajasthan, *Luffa acutangula* and *Luffa cylindrica* are majorly found in the places like Baran, Jodhpur, Tonk, Ajmer, Bharatpur, Ganganagar and Jaipur. Sowing of *Luffa* seeds is done mostly in July and it takes 3-4 months to produce unripe fruits.. The fruits and seeds are used in herbal preparations for the treatment of venereal diseases, particularly gonorrhoea. They are used in Vatta, Kapha, Anaemia, Asthma, Leucoderma, Tumors and Jaundice. Fruits are also useful as diuretic and in splenic enlargement. Plant grows very well in a warm hot climate, the optimum temperature being 25°–30°C. The ridged gourd contains protein, fats, carbohydrate and many micronutrients.

Luffa cylindrica is widely distributed in the tropics and subtropics, as a cultivated and naturalized plant. It is the most important medicinal plant and has been used in various disorders. *Luffa cylindrica* is the rich source of antimicrobials, flavonoids and sterols. The seeds of both above species yield an edible oil that is, however, sometimes

bitter and toxic. The bitter and toxic seedcake is unsuitable as feed for cattle, but can be used as fertilizer given that it is rich in nitrogen and phosphorus.

Luffa acutangula and *Luffa cylindrica* are attacked by many fungal diseases like powdery mildews, downey mildews and anthracnose and many other plant pathogens like Alternaria, Phytophthora, Plasmopara etc which are controlled by fungicides. Fungicides in powdered form are around 90 % sulphur that is very toxic. Aiming to minimize the negative effect of fungicides, there has been development of the alternative control of plant diseases which includes the biological control, the induction of resistance and use of natural products with induction of resistance with direct antimicrobial activities. These include use of extracts and essential oil from various plants . Plant extracts as natural products are much safer than conventionally used fungicides.

Importance of Proposed Investigation

All plant diseases result from a three-way interaction between the host, the pathogen and the environment. An epidemic develops if all three of these factors are favorable to disease development. Therefore, disease can be controlled by manipulating one or more of these factors so that conditions are unsuitable for replication, survival or infection by the pathogen. Pathogens can be controlled by Quarantine, Chemical Management or by Biological Control.

It is found that fungal infection, insect infection and pest infection harm the agriculture field at vast level. To protect the crops from these infections, farmers have to use fungicides, insecticides and pesticides, and consequently the environment gets polluted. The pH level of soil also gets disturbed and soil get infertile in nature. Humankind and animals are also affected adversely. Humans suffer from diseases like Asthma, Eyes infection, Sinus, Skin allergy, Cancer, Tumors, Leucoderma etc. The pH level of soil also disturbed and soil get infertile in nature. To maintain the nature healthy in all respect, it is important to investigate scientifically medicinal plants which have been used in traditional medicines as potential source of antimicrobial compounds.

In recent years, plant based essential oils and extracts were focused. The main role of medicinal plant extracts and oil is to control the population of pathogens in agriculture due to its antimicrobial properties. Many medicinal plants contain alkaloids in nature. Plants are rich in secondary metabolites having important source of micribicides and pesticides (**Archana *et al.,* 2011**). Plant based fungicides are easily biodegradable in nature so it will not harm the soil pH level and its fertility.

The present problem deals with the plants that may contain secondary metabolites which can effectively control pathogens. Biofungicides made from these, plant extracts will be safe, biodegradable and nontoxic.

Objectives

1. To identify fungal diseases of leaf of locally grown *Luffa acutangula* and *Luffa cylindrica* on the basis of morphology and measurement of hyphae and conidia.
2. To isolate, purify and identity fungal diseases of leaves of *Luffa acutangula* and *Luffa cylindrica*.
3. Selections of few weed plants available locally, for study against pathogenic microorganisms.
4. Screening of photochemical and antioxidants from weed plants.
5. Comparative study of measurement of zone of inhibition (in mm.) by extracts of weed plants with commercially available fungicides.

Review of Literature

Trease and Evans (1983) stated that nature is an immense source of pharmacologically active molecules which has been used for the treatment of the incurable diseases without any adverse effects.

According to **Chiang *et al.* (2004)** natural antioxidants counteract and detoxify reactive oxygen species (ROS) in order to survive and appear to contribute chemo protective activity by maintaining homeostatic balance between reactive oxygen species and endogenous antioxidants.

Studies of **Zayachkivska *et al.* (2005)** showed that flavonoids and seed extracts possesses significant antioxidant activity and appears to be associated with a reduced risk for certain chronic diseases, such as cancer, inflammation and ulcer.

Du (2006) has carried out Hydroplilic antioxidant constituents in the fruits of the vegetable *Luffa cylindrica* which were separated by an antioxidant guided assay which was evaluated by radical scavenging effect on the DPPH radicals and concluded that the consumption of sponge gourds can supply some antioxidant constituents to human body.

According to **Mustafa and Thunibat (2008)** indian system of medicines has been recommended for different kind of biological activity for their health care needs.

Oboh and Aluyor (2009) has undertaken *Luffa cylindrica* in his work as an emerging cash crop and resulted *Luffa cylindrica* encourages the commercial cultivation of the crop of its increasing economic importance and showed the potentialities of this crop that is virtually found around the globe.

Oboh *et al.* (2009) has carried out Post treatment of produced water before discharge using *Luffa cylindrica* and concluded *Luffa cylindrica* is an effective biosorbent for the removal of heavy metal ion and offered a cheap option for primary treatment of produced water.

Muthumani (2010) has carried out the Phytochemical screening and Anti inflammatory, Bronchodilator and Antimicrobial activities of the seeds of *Luffa cylindrica* and concluded that all the extracts revealed the presence of sugar, protein, alkaloids, flavonoids, sterols and glycosides as major constituents.

Dried seeds of *Luffa cylindrica* were extracted by continuous hot percolation method using solvents of increasing polarity from petroleum ether to ethanol which was carried out by **Susiuthra *et al.* (2010)** to evaluate anti-inflammatory activity of seeds of *Luffa cylindrica* by using carrageenan induced paw oedema method comparing with marketed available diclofenac sodium gel.

Nithya and Bhaskar (2011) analysed that ayurvedic plants are more potent and known to have human medicinal properties. **Nenaah and Ahmed (2011)** showed that the products obtained from the plant source are clinically used for health benefits without any chemical modification. **Joseph and Raj (2011)** concluded that more than 21000 plants have been used as medicinal agent around the world**. Archana *et al.* (2011)** surveyed that there are lots of plants to be explored for their pharmacological activity**. Karim *et al.* (2011)** stated that an 80% of the world population employs herbal medicines to deals with their daily medicinal issue and they have negligible adverse effect as compare to modern medicine. **Oladosu *et al.* (2011)** analysed that plants have

great value to phytochemists because of their medicinal properties**. Khasawneh *et al.* (2011)** stated that phytoconstituents are the immense source of natural antioxidants.

Dandge *et al.* (2012) have done Pharmacognostic study and Antimicrobial activity of *Luffa acutangula* on some Deuteromycetes Fungi. It was found that Fruit extract of *Luffa acutangula* are more potent antibacterial and antifungal activity than leaf extract. *E.coli* showed high sensitivity than *Staphylococcus aureus, Pseudomonas aeroginosa* species to leaf and fruit extract of *Luffa spp*. Among fungi *Curvularia lunata* was found highly sensitive to leaf and fruit extract of *Luffa spp.* while to same extract *Phoma sorghina* showed poor sensitivity.

Rathi *et al.* (2012) evaluated the antifungal and antibacterial activity on naturetic (Herbal) plants. Agar tube dilution for antifungal, Agar diffusion assay for antibacterial as per Protocol was conducted to determine the Zone of Inhibition. The different concentration of various plants extracts were tested on microorganism for the Zone of inhibition. It showed that water extract of *Sapindus emarginatus* and *Mirabilis jalapa* reported the highest inhibition against the bacterial organism at 20mm- 23mm and 16mm-19mm against the fungal strains. The methanol extracts of *Hibiscus rosa-sinensis, Vitex negundo* , *Saussurea lappa Costus* reported the highest inhibition against the bacterial organism at 23mm- 26mm and 17mm-20mm against the fungal strains. The n-Hexane extracts of *Euphorbia tirucalli* reported highest inhibition at 21mm- 24mm and 16mm-19mm against the fungal strains. This study concluded that all solvents showed the high zone of inhibition against all different extracts of Plants except chloroform .

Sadda and Varma (2012) studied the infection on *Luffa cylindrica* seeds In dry seed examination black pin head like minute spots (microsclerotia) of Rhizoctonia solani were reported which covered the seed surface of about 99 samples out of 120 seed samples of sponge gourd belonging to 7 areas of Kota district viz. Arjunpura, Arandkhera, Giridharpura, Kethun, Raipura, Nayanohra and Dhakarkheri of Rajasthan. Incubation of these symptomatic seeds yielded pure growth of the pathogen. The percent range of fungi associated with such seeds varies between (2.25-35.25%). Weakly symptomatic seeds carried 1-10% sclerotia whereas heavily infected seeds were sparsely to fully covered with sclerotia.

According to **Sarin and Samariya (2014)** *Luffa cylindrica* and *Citrullus colocynthis* are rich source of antimicrobials, flavonoids and sterols.

Research Gaps identified in the proposed field of investigation

1. Fungal diseases cause severe damage and in case of *Luffa spp.* this causes approximately 30-40 % post harvest loss. To reduce this loss several synthetic fungicides are indiscrimately used by the farmers.
2. Use of fungicides, pesticides and insecticides cause harmful effect on the environment, soil, food material and fauna of soil. In totally it causes environmental pollution , a root cause of various diseases in human being.
3. Another method of management of diseases control is biocontrol but no literature is available on herbal control of the diseases of *Luffa acutangula* and *Luffa cylindrica* . Studies on the biocontrol of these plants are scarce. Reviewed literature showed that work on fungal control of *Luffa spp.* through fungicides is in use but no work on control through phytochemical is done.
4. So there is lacuna in research as far as biocontrol by biofungicides are concerned and further research is possible to inhibit the fungal diseases by plant extract.
5. Antioxidants and phytochemicals present in extract of weed plants will be employed to control the pathogen. Hopefully that will help to fill the research gap.

Major Hypothesis

Firstly, the intensive and indiscriminative use of fungicides has caused many problems to the environment as they are not biodegradable.

Secondly, since most of the pathogenic microbes have developed resistance against chemical drug derivatives, the need of the hour is to develop or formulate plant based natural biofungicide. These formulations with less or no toxicity can prove a better alternative of fungicides. Weed plants as potential biocontrol agent can be further proceeded for commercialization.

Research Methodology

The proposed plan of work is given below. The appropriate methods will be adopted for the identification of fungal diseases of leaf and Phytochemical analysis. Department of Botany, Government College, Ajmer is equipped with of all facilities of microbial cultures and for related experiments.

1. Collection of leaf fornightly of *Luffa acutangula* and *Luffa cylindrica* will be done in sterilized polybags from different localities of Ajmer.

2. Fungal diseases of leaf will be isolated on Potato Dextrose Agar (Ricker and Ricker,1936), Czapek Dox Agar (Thom and Raper, 1945) and Sabouraud Dextrose Agar (Sabouraud,1892).

3. Purification and identification of fungal diseases of leaf will be carried out according to the morphology and measurements of hyphae and conidia. Gaumann (1928) , Barnett (1960), Ellis (1971), Clements (1973).

4. Pure broth culture will be prepared under aseptic condition.

5. Approximately 2 ml – 3 ml of broth culture will be poured and evenly spread in Potato Dextrose Agar, Czapek Dox Agar and Sabouraud Dextrose Agar plates.

6. Screening of antifungal sensitivity using agar well diffusion method (Omenka and Osuoha , 2000) will be done.

7. Weed plants such as *Calatropis procera , Argemone maxicana , Withania somnifera , Lantana indica* and *Jatropha gossypifolia* will be shade dried for their antifungal

sensitivity test and their extracts like alkaloids, flavnoides , sterols and glycosides will be prepared in partially purified solvents like distilled water, methanol and ethanol etc.

8. Aliquot of plant extract solution will be poured in well made in potato dextrose agar, Czapek dox agar and Sabouraud dextrose agar plates.

9. Zone of inhibition will be measured in millimeter (in mm).

10. Comparison of these will be done with commercially available fungicides.

11. Phytochemical screening will also be done .

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Signature of the candidate with date

Outline approved

Name and signature of supervisor with date and seal

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(Dr. Mamta goyal)

To,

Registrar

Respected Sir/ Madam,

With due respect it is bring to your kind notice that I, Deepika Chittoriya is submitting the Revised Synopsis after rectification.

Supervisor **Head of the department**

Vice Principal